Graphene-Based Bionic Composites with Multifunctional and Repairing Properties

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ABSTRACT: In this work, a novel bionic composite inspired by the concept of yeast fermentation has been proposed. It was observed that the addition of graphene nanoplatelets during the fermentation of extract of Saccharomyces cerevisiae fungi allows coupling of the graphene sheets to the yeast cell wall. This process resulted in the formation of a composite film with improved mechanical and electrical properties along with the capability of converting the light stimulus in the electrical signal. The mechanical properties of the prepared composites, namely, the fracture strength and Young’s modulus, were studied via numerical simulations and are related to the properties of the constituent phases via rules of mixture. Finally, it was observed that graphene nanoplatelets, added to the nutrient broth, were able to reassemble onto the stressed cell surface and repair the surface cracking, partially restoring the pristine electrical and mechanical properties. The method reported here may find potential application in the development of self-healable bioelectronic devices and microorganism-based strain and chemical biosensors.

KEYWORDS: graphene, biohybrid composites, functional properties, self-repairing

Unicellular microorganisms, such as bacteria and fungi, are extensively used in materials science as simple templates with monodisperse sizes and shapes as well as low cost and scalable growth.1 The features of a living cell along with characteristics of inorganic nanoparticles can be used for the development of novel materials. For example, encapsulation of the cell with inorganic shell has been found to enhance the robustness of the cell and improve its endurance in harsh environments. Some materials such as silica,2 calcium phosphate,3 and calcium carbonate4 have already been used as encapsulating materials to improve the cell stability.

Moreover, the cell wall can be considered as an effective and cheap scaffold for the deposition of nanomaterials with practical application in microelectronic devices such as electronic conductive microbridges and electrochemical biosensors.5

It was found, for example, that immobilization of hydrophilic oxidized carbon nanotubes onto the oppositely charged polyelectrolyte-coated yeast cells indicates that the polyelectrolyte/nanotube coating affects the electron mediation between yeast cells and an artificial electron acceptor.6 This effect has been used to distinguish living and dead cells in various tests.

Among nanostructured carbon materials, we focused our attention on graphene because of its high electrical and mechanical properties and large surface area.7 Interfacing living cells with graphene has been proven to be useful for the integration of cellular physiology with electrical readouts.8 In the past, several studies reported the interaction of the edges of graphene sheets with the cell wall membrane,9 the generation of reactive oxygen species by graphene,10 and the trapping of...
living cells within graphene sheets. Most recently, Kempaiah et al. encapsulated yeast cells with hard shells of graphene sheets, leading to enhanced protection of the cells from osmotic stresses and improving their stability, while Yang et al. formed a nanosheet of graphene oxide on individual yeast cells by selectively depositing graphene oxide via layer-by-layer self-assembly.

These previous attempts considered abiotic processes, where coupling between the inorganic phase and living cell was obtained by chemical methods; here we propose a novel biogenic technique that exploits the ability of a microorganism to digest nutrients and use the byproduct as coupling agents with the inorganic phase. *Saccharomyces cerevisiae* (SaC) is a type of yeast widely used in the conversion of sugar and starch-based substrate into ethanol because of its ability to produce high amounts of ethanol and glucose as byproducts. Moreover, in brewer yeast fermentation flocculation commonly occurs when the sources of fermentable sugars are exhausted. During flocculation, the yeast cells aggregate rapidly in the medium in which they are suspended, forming a film after removal of the liquid medium.

In this paper, we report a method where the production of glucose during SaC fermentation was used to couple graphene nanoplatelets (GNPs) onto the yeast cell wall and, when sugar is exhausted, to obtain composite films with synergetic mechanical and electrical properties. Finally, from this...
fermentation process, in the presence of a nutrient material, i.e., sucrose, the yeast cells trigger the graphene assembly that is used to self-repair the mechanically damaged composite.

For preparation of the composite film, 40 mg of GNPs (purchased from Cheaptubes; thickness 8–15 nm) was dispersed for 3 h at room temperature in 40 mL of water using a sonication bath. A SaC-based commercial beer yeast extract with additives was used as the medium for fermentation. The dispersion of GNPs (1 mg/mL) was then added to the yeast solution and stirred at 110 rpm at 30 °C for 1 h. To start the fermentation, sugar (i.e., sucrose, 4 g) was added to the yeast/GNPs solution. The dispersion was heated at 35 °C to allow the fermentation process to proceed. The solid films were obtained by evaporating the water in excess, leaving the solutions in a sterilized silicon rubber mold at 30 °C in an inert atmosphere for 2 nights. Field-emission scanning electron microscopy (FESEM) was used to investigate the cross sections of the samples obtained by fracture in liquid nitrogen. Ultraviolet–visible (UV–vis) measurements of the deposited films were carried out with a PerkinElmer spectrometer Lambda 35; for all samples, a neat quartz slide was used as the reference. The optical absorbance was obtained on films of the same thickness (∼5 μm). The current–voltage (I–V) characteristic was performed by a computer-controlled Keithley 4200 Source Measure Unit. The electrical conductivity of the samples was monitored, at room temperature, by applying a sweeping direct-current electric voltage across the sample. The photocurrent was recorded on the same samples as those prepared for the optical absorbance, under an AM1.5D 150 mW cm−2 illumination source from a Thermal Oriel solar simulator. Photoelectrical measurements were obtained for the films over several on/off light illumination cycles.

FESEM was used to investigate the surfaces of the samples. Parts a and b of Figure 1 show that the fermented yeast film mainly consist of whole cells and that the plain yeast cells do not exhibit surface features. The effect on the film morphology when GNPs were added during fermentation is reported in Figure 2. The line scan on the GNP/yeast cell surface indicates for the composite film the formation of wrinkles; these wrinkles arise from compressive stress when a soft-matter core (i.e., SaC yeast cell in our case) is coupled with a thin, high modulus sheet (i.e., GNPs).17

According to Cerda and Mahadevan,17 in the case limit when the wavelength of the wrinkle is much lower than the substrate thickness and much higher than the skin thickness, the physics and geometry of such a system indicates that the wrinkle wavelength is given by:

\[ \lambda = 2\pi \left[ \frac{(1 - \nu_s^2)}{\left(1 - \nu^2 \right)} \frac{E}{3E_s} \right]^{1/3} \]

where \( \lambda \) is the thickness of the GNP skin, \( E \) and \( \nu \) are respectively Young’s modulus and Poisson’s ratio of the skin, and \( E_s \) and \( \nu_s \) are respectively Young’s modulus and Poisson’s ratio of the substrate. Assuming an average thickness \( t = 11.5 \) nm, \( E = 50 \text{ GPa} \) as determined below, \( \nu = 0.165,20 E_s \approx 112 \text{ MPa}^{21,22} \) and \( \nu_s = 0.522 \) a wrinkling wavelength \( \lambda = 38 \) nm would be obtained. This result is in agreement with our experimental value of 30–70 nm reported in Figure 2b.

The tensile properties of films, i.e., Young’s modulus (E) and failure strength (\( \sigma_f \)), were then measured using a universal tensile testing machine (Lloyd Instruments LR30K) with a 50 N static load cell. The film samples were cut into strips (30 mm × 12 mm). The gauge length was 20 mm, and the extension rate was set at 2 mm/min. \( \sigma_f \) is determined as the peak stress in the measured stress–strain curve, while the Young’s modulus is evaluated as the secant between strains of 0.1% and 0.2%. The mechanical and electrical properties of the plain yeast film (Figure 1c,d) were found to be improved when the cell wall was coupled with GNPs (Figure 2c,d). Such results will be rationalized below according to a synergetic effect.

Light absorbance in the UV–vis range of the prepared samples is presented in Figure 3a. In the visible range (350–800 nm), depending on the GNP incorporation, differences were obtained in terms of normalized optical absorbance. Figure 3b shows that a photocurrent signal was recorded on both samples. The spectral irradiance for a xenon lamp between 400 and 500 nm under the AM1.5D standard condition is about 16.9%; the absorption spectrum of the plain yeast film (Figure 1c,d) were found to be improved when the cell wall was coupled with GNPs (Figure 2c,d).
disturb the cell proliferation and that, at the same time, are able to reassemble onto the cell surface when encapsulation is broken by an external harsh environment. Once shell structures are broken, a decrease of the mechanical strength and a loss of functionality of encapsulated cells will occur. A way to self-repair the functional nanoscale shell is thus preferred, mainly if such nanoshells could self-assemble onto the cell surface during its proliferation. The repairing properties of our biohybrids are reported in Figure 4. After 30 min in the high-vacuum conditions of FESEM (Figure 4a), the presence of cracks on the surface of the cell is well visible and suggests that a strain could induce such an effect on GNP sheets. It could be reasonable that coupling between the GNP sheets and the cell wall hinders cell dehydration through its membrane, and the yeast cell would expand its volume via hypoosmotic shock. This straining effect is evident on the mechanical and electrical characteristics of the graphene-based composite, as reported in Figure 4c−e, where the decrease of the electrical conductivity as well as the reduction of the mechanical strength with respect to the unstressed composite film is attributed to the loss of the graphene connecting network.

We observed that such an effect is reversible upon placement of the composite film in the nutrient broth, i.e., an aqueous solution of sucrose and GNPs, for 40 min. As suggested by FESEM analysis (Figure 4b), the cracks almost disappear; the nutrient broth reconstructs the GNP shell, and the strain-induced effect on GNP sheets is canceled, with the mechanical strength and electrical conductivity being almost restored (Figures 4d−f).

The bionic composite shows remarkable improvements in the mechanical properties. Indeed, the undamaged composite has 3.5 times higher failure strength, and it is about 9 times tougher than the pristine yeast. Even the damaged film is able to guarantee an enhancement in performance, being 1.5 times stronger and showing an increase of toughness of +29%. After the self-repair, the composite is able to restore 85% of the undamaged composite strength and 83% of the toughness, thus showing a quite efficient healing mechanism.

The measured elastic and mechanical properties of the bionic composites, namely, the fracture strength $\sigma_f$ and Young’s modulus $E_f$, can be related to the properties of the constituent phases via rules of mixture:

$$\sigma_f = f \sigma_{GNP} + (1 - f) \sigma_{yeast} \tag{2a}$$

$$E_f = f E_{GNP} + (1 - f) E_{yeast} \tag{2b}$$

where $f$ is the volumetric fraction of the GNP film and the subscripts refer to each component of the bionic composite. A simple system of two fermented cells was modeled via Finite Element Method (FEM) simulations in order to understand the effect of the incorporation of GNPs. As operated in our work on yeast carbon nanotubes bionic composites, the shape of the cells within the ensemble is approximated as hexagonal prism, starting from the actual cell arrangement in the ensemble.
A slight difference in the mechanical properties was reported,23 with the latter being softer. Actually, the experimental stress-strain relationship can be considered an average of the two. The constitutive behavior of graphene has been taken from Xu et al.,20 operating a scaling on the failure strength of the curves there reported in order to best fit the experimental curves with simulations and thus have an estimate of the mechanical properties of GNPs. Perfect bonding was assumed at the interface between the GNPs film and cell wall, with the constitutive behavior of this interface being unknown.

Figure 5 shows a comparison between the curves derived from experiments and from simulated tensile tests on two cells. The overall trend is well comparable, and the mechanical parameters determined from the simulation best fit are comparable with the one estimated with rules of mixture (Table 1), confirming the synergetic interaction between the GNP film and yeast cells. Our back-analysis estimation for the GNP mechanical properties is consistent with the literature.27 The fracture pattern within the graphene film is also depicted (Figure 4b). The dimensions of the cells, mother and daughter, were taken from the work of Ahmad et al.25 They reported for the mother cell a diameter \( d_{\text{m}} = 5.565 \, \mu m \) and for the daughter cell \( d_{\text{d}} = 4.467 \, \mu m \). The cell dimension measurements from this work are consistent with our estimations.24 The hexagonal base of the prism was dimensioned in order to have an area equivalent to a circle of diameter \( d \), while the cell height was finally univocally determined from the cell volume also reported elsewhere.25 Considering these geometrical characteristics for the cell, we obtain that \( f = 0.35\% \) and \( 0.18\% \), assuming limit values for the GNP film thicknesses of \( t_{\text{GNP,max}} = 15 \, \text{nm} \) and \( t_{\text{GNP,min}} = 8 \, \text{nm} \). The corresponding average for \( t_{\text{GNP,av}} = 11.6 \, \text{nm} \) is \( f = 0.25\% \). With regard to the yeast constitutive behavior, the curve obtained from the pristine yeast (Figure 1c) has been adopted as input for simulations, using an elastic material with finite strain capability. The same relationship was same in the mechanical properties was reported,24 with the latter being softer. Actually, the experimental stress–strain relationship can be considered an average of the two. The cells are modeled with underintegrated solid elements with spurious mode stabilization.26 The load is applied as imposed displacements on the lateral face of the cells (Figure 5). The interface between the two cells was modeled via a cohesive-zone-model-based contact28 for which the force–separation curve was derived in a study by the authors and associated with an estimated fracture energy of \( G_{\text{fract,pristine}} = 0.0193 \, \text{N/m}^{2} \).25 The GNP film has been modeled with shell elements with a thickness of 11.5 nm, being the average of the expected film thickness. This corresponds to a total of 34 layers of graphene, each of them associated with a through-thickness integration point within the shell. Two half cells with a GNP film in between has been modeled in a sort of “sandwich” structure with periodic boundary conditions at the symmetry planes: by doing this, we simulate an infinite multilayer alternation of the yeast cell layer and GNP film (Figure 5). The constitutive behavior of graphene has been taken from Xu et al.,20 operating a scaling on the failure strength of the curves there reported in order to best fit the experimental curves with simulations and thus have an estimate of the mechanical properties of GNPs. Perfect bonding was assumed at the interface between the GNPs film and cell wall, with the constitutive behavior of this interface being unknown.

### Table 1. (a) GNP Properties Determined from Experiments through Rules of Mixture and from Numerical Simulations

<table>
<thead>
<tr>
<th>material</th>
<th>( \sigma_0 ) [MPa]</th>
<th>( E ) [MPa]</th>
<th>( \sigma_{\text{GNP}} ) [MPa]</th>
<th>( E_{\text{GNP}} ) [GPa]</th>
<th>( \sigma_0 ) [MPa]</th>
<th>( E ) [MPa]</th>
<th>( \sigma_{\text{GNP}} ) [MPa]</th>
<th>( E_{\text{GNP}} ) [GPa]</th>
</tr>
</thead>
<tbody>
<tr>
<td>yeast + GPNs</td>
<td>3.1</td>
<td>159</td>
<td>632–1185</td>
<td>33.9–63.6</td>
<td>3.2</td>
<td>135</td>
<td>882</td>
<td>50.1</td>
</tr>
<tr>
<td>yeast + GPNs dam.</td>
<td>1.3</td>
<td>54</td>
<td>136–255</td>
<td>3.5–6.6</td>
<td>1.2</td>
<td>50</td>
<td>126</td>
<td>5.3</td>
</tr>
<tr>
<td>yeast + GPNs rep.</td>
<td>2.6</td>
<td>115</td>
<td>500–937</td>
<td>21.2–39.7</td>
<td>2.6</td>
<td>107</td>
<td>670</td>
<td>31.7</td>
</tr>
</tbody>
</table>

*The two values for the experimental estimates of the GNP properties come from the assumption of the GNP film thicknesses of \( t_{\text{GNP,max}} = 15 \, \text{nm} \) and \( t_{\text{GNP,min}} = 8 \, \text{nm} \), respectively. For FEM simulations, an average thickness of \( t_{\text{GNP}} = 11.5 \, \text{nm} \) is assumed.*
the cell wall. The method presented here may cells is important for creating fast and cheap sensors for the nanoshells that correlate the mechanical strain with the synergetic mechanical coupling with the cell. The described electrically conductive layer on the yeast cells, developing a sucrose. We demonstrate that graphene sheets form an self-healable nanoshells that correlates the mechanical strain with the electrical readout. The possibility of distinguishing stressed cells is important for creating fast and cheap sensors for the detection of osmotic stress or testing nutrient adsorption on the cell wall. The method presented here may find a wide range of applications in bioelectronics and the development of novel materials.

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L.V. and N.M.P. had the idea, designed and supervised the entire research, and analyzed the results. S.B.B. prepared the samples and performed the characterizations. S.S. performed the FEM simulations. All authors have revised and given their approval to the final version of the manuscript.

Notes
The authors declare no competing financial interest.

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